Research Article

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Investigation on *Trifolium Prantese* Capped ZnO Nanoparticles for Cancer Applications

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ABSTRACT

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience. In the present study, ZnO nanoparticles were synthesized with and without Trifoliumprantese flower extract by sol-gel assisted microwave irradiation method. The synthesized nanoparticles were characterized with various techniques such as XRD, FTIR, SEM, and EDAX. The formation of zinc oxide nanoparticles has been characterized by X-ray Diffraction (XRD). The elemental composition of the prepared sample was analyzed using Energy Dispersive X-ray analysis (EDAX). The surface morphological structure of zinc oxide nanoparticles was obtained from Scanning Electron Microscope (SEM). The functional groups of the ZnO are investigated through Fourier Transform Infrared spectroscopy (FTIR). The zinc oxide nanoparticle (ZnO) is mostly applied in the field of medical, electronic devices, biosensors, and anti-microbial agents.

Keywords: Green Synthesis; Microwave irradiation method; Trifolium prantese; Zinc oxide.

1. INTRODUCTION

Nanotechnology is increasingly gaining traction in areas such as medicine, agriculture, and physics. The synthesis of nanoparticles (NPs) for the development of new smart materials with unusual properties at the nanoscale has increased dramatically in recent years (Albrecht et al. 2006; Singh et al. 2016). Metal NP synthesis is a common topic in nanoscience these days. Several scientific groups have been interested in metal NPs such as iron oxide, silver nitrate, copper oxide, and zinc oxide over the last few decades (Rouhi et al. 2013; Tiwari et al. 2018). Metal NPs are synthesized using a variety of processes, including the sol-gel process, thermal decomposition, hydrothermal, microwave irradiation, among many others (Kolekar et al. 2013). However, owing to the production of a large volume of secondary waste materials as a result of the addition of chemical agents for the reduction process, these chemical and physical synthesis approaches are time-consuming, expensive, and harmful.

As a result, biological NP synthesis would be a viable alternative for reducing toxicity, cost, and time. In comparison to physical and chemical approaches, biological synthesis of NPs from plant extracts has been identified as a superior option. Biologically synthesized NPs are biocompatible and non-toxic, and they are used as drug carriers and fillers in medical products (Rosi and Mirkin, 2005). Several studies based on green synthesis of ZnO NPs of various plants extract have exists, such as *Cassia tora* L. (Manokari and Shekhawat, 2017), *Sageretia thea* (Khalil *et al.* 2017), *Calotropis gigantean* (Chaudhuri and Malodia, 2017), *Azadirachat indica* (Bhuyan *et al.* 2015), *Hibiscus rosa-sinensis* (Bala *et al.* 2015), *Ocimum basilicum* L. *var. Purpurascens* (Bi *et al.* 2017), *Corymbia citriodora* (Zheng *et al.* 2015), *Zingiber officinale* (Raj and Jayalakshmy, 2015), and *Anisochilus carnosus* (Anbuvannan *et al.* 2015).

Trifolium prantese, also known as red clover, is a flowering plant of Indian origin that belongs to the Fabaceae tribe. It is a well-known medicinal plant with a high concentration of secondary metabolites (Don, 1999), with over 200 terpenoid-based indole alkaloids found in different plant parts such as the leaf, stem, vine, and root. These alkaloids are responsible for anticancer, astringent, antibacterial, anti-diabetic, anti-fungal, and anti-malarial effects, among others (Noble, 1990). The alkaloid content of C. roseus varies considerably in various parts, the maximum being in the root bark, which ranges from 0.15 to 1.34 % and even up to 1.79 in some Strains. The 1 plant contains about 130 alkaloids of the indole group, out of which 25 are dimeric. Two of the dimeric alkaloids vinblastine and vincristine, mainly present in the aerial parts, have found extensive application in the treatment of human neoplasma. The reports published on the green synthesis of ZnO NPs using flower extract of *T.prantese* are very few (Bhumi and Savithramma, 2014; Kalaiselvi *et al.* 2016) with a fragmented knowledgebase. Green ZnO NPs were synthesized, characterized, and their antimicrobial activities were assessed in this report. During the research, the synthesized NPs were shown to have a synergistic interaction with pre-existing antibiotics.

T.prantese flower extract was used in this analysis to biosynthesis ZnO NPs under various physical conditions. SEM (Scanning Electron Microscopy), EDAX (Energy Dispersive X-ray Analysis), FTIR (Fourier Transform Infrared Spectroscopy), and XRD were used to classify the synthesized ZnO NPs (X-Ray Diffraction).

2. MATERIALS & METHODS

2.1 Green Synthesis of ZnO

Zinc can be found in ocean water, and the air and sodium hydroxide (NaOH) were received from India. *Trifolium prantese* flower (Fig 1) was collected from the surrounding area and washed several times using running tap water and then again washed double distilled water to remove dust particle then dried to remove residual Moisture. Taken 50g of the flower were mixed with 150ml of distilled water. This was boiled at 75ºC for 30 minutes. The extract was filtered using whatmann No.1 filter paper to get a clear solution. In this method, 10.10g of Zinc Acetate was dissolved with 100ml of distilled water, and it was stirred for about 30 minutes. After that 10ml of flower extract was added drop-wise into the above solution. The pale green color solution was obtained. The mixture was stirred for 45 minutes. During this process, the NaOH solution was added drop-wise to maintain the pH as 12. The gelatinous precipitate was continuously stirred for 1hours. It was aged 24 hours at room temperature. Then the precipitate was washed once with distilled water to remove impurities. To attain the minimum time consumption microwave oven was used to dry the precipitate at 70W for 35 minutes. Finally, the dried sample was grinded by using mortar to get *Trifolium prantese* flower capped ZnO Nanoparticle. Further, Zinc Acetate with no flower extract was synthesized and named as pure ZnO (PZnO) for assessment.

2.2 Characterization Techniques

2.2.1 FTIR

The functional groups of prepared samples were identified using Fourier transform spectroscopy analysis. The spectrum was recorded in the range of 4000-400 cm^{-1} region.

2.2.2 XRD

The prepared samples were analyzed using XRD (X – ray Diffraction) technique. This XRD pattern predicts the lattice parameter (a and c), unit cell volume, and crystalline size of the sample. The XRD pattern of prepared samples was well-matched with JCPDS card no: 09-0432(corresponding to hexagonal phase). The lattice parameter of the sample was calculated using the following equation:

$$1/d^2= (4(h^2+hk+k^2)/3a^2) + (1^2/c^2))$$

Where, d is the spacing between the planes, a and c are the lattice parameter. The unit cell Volume (V) of the sample was described using the given equation:

$$V= (\sqrt{3}/2) + a^2+c^2$$

The average crystalline size of the sample was determined by using the Scherrer's formula.

$$D =k\lambda/\beta \cos \theta$$

Where D denotes the average crystalline size of the sample, K represents the broadening constant, λ denotes the wavelength of CuKλ radiation source (1.54Å), β represents the full width at half maximum, and angle of diffraction is denoted by θ.

Fig. 1: ***Trifolium prantese*** **Flowers**

2.2.3 SEM & EDAX

The surface morphologies of synthesized ZnO samples were analyzed using Scanning Electron Microscopic analysis (SEM). Energy dispersive spectroscopy is used to identify the elemental composition of the sample.

3. RESULTS

3.1 XRD Analysis

XRD pattern of ZnO nanoparticles was calculated using x-ray diffraction method. The most prominent peaks of the ZnO samples illustrated in (Fig. 2) were observed. The XRD pattern of wurtzite hexagonal structure of ZnO nanoparticles has been verified with JCPDS file number: 36-1451 and it is well matched. The lattice constant a and c, unit cell volume (V) were calculated using the standard equation. For PZnO the average crystalline size was 18.67nm, and for GZnO 15.58 nm was shown in (Table 1). The capping of flower extract enhance the property by decreasing the crystalline size in the sample. The diffraction peaks of the prepared PZnO and GZnO at 2θ values, corresponding to (101), (103), (112), and (201) hkl planes, respectively.

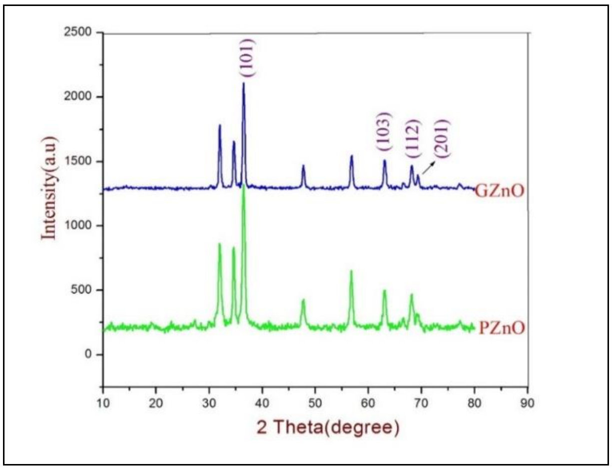

Fig. 2: Synthesized Sample with XRD Pattern for PZnO and GZnO

Table 1. XRD Pattern of Synthesized PZnO and GZnO Nanoparticles

Sample name	2θ (deg)	FWHM (deg)	d (Å)	Intensity (Counts)	Crystalline Size(nm)	Average Crystalline size	Hkl	Lattice constant		Unit cell volume (V)
								a= b	c	
PZnO	36.33 63.03 68.12 69.29	0.4703 0.5020 0.5205 0.4849	2.46 1.47 1.37 1.35	1195 358 292 163	17.78 18.86 18.42 19.91	18.67	101 103 112 201	3.22	5.20	PZnO
GZnO	36.42 63.00 68.11 69.24	0.5609 0.5807 0.6641 0.6759	2.46 1.47 1.37 1.35	739 210 177 84	17.57 14.42 16.09 14.27	15.58	101 103 112 201	3.22	5.23	46.51 48.39 46.51 46.80

3.2 FTIR Analysis

FTIR spectrums of the prepared ZnO samples were recognized using at a wavelengthrange of 4000–400 cm^{-1} is shown in (Table 2). The observed peak resulted from the chemical Synthesis method is at 3861.49 to 879.54cm^{-1}, whereas from the green synthesis method, the peak is observed at 3960.65 to 570.92 cm^{-1}. The vibrations of a variety of groups are present at different wavenumbers of IR radiation. The broad peak was absorbed at 3861.49 cm^{-1} and 3960.65 cm^{-1} (Alcohol) which be in contact to O-H stretching band. C-H stretching confirms from the absorption peak of 2800.64cm^{-1} and 2809.44cm^{-1} (Alkynes).N=O stretching from the absorption the peaks at 1450.47 cm^{-1} and 1450 cm^{-1} (Nitro). The FTIR spectrum absorbs the peak at 3589.53cm^{-1} and 3446.81cm^{-1} were calculated with the stretching vibrations of N-H(Amine) bond. Introducing a capping agent has created a minor change in the functional group analysis of the samples. The spectrum (Fig. 3) reveals the FTIR graph of PZnO and GZnO.

3.3 SEM & EDAX

The scanning electron microscope (SEM) analysis was performed to determine the shape and Morphology of ZnO nanoparticles under different magnifications. The SEM results of the studied ZnO nanoparticles synthesized using both chemical and green techniques. It can be seen from (Figure 4) illustrated morphological descriptions and elemental composition of PZnO and GZnO. Which shows Needle shaped morphology for PZnO and Spherical shaped morphology for GZnO, which present capping agent can create in a change in morphology of the sample. The average grain size was found around approximately, 27to 42nm for PZnO .and 70 to 84nm for GZnO.

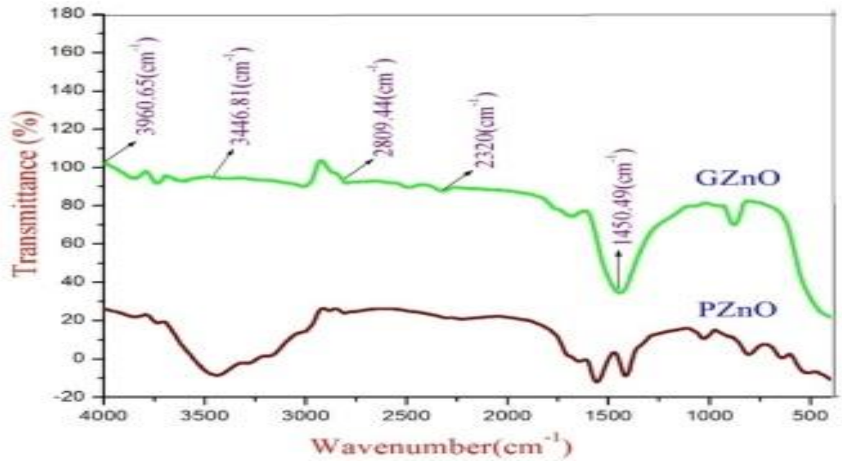

Fig 3: The FTIR spectrum of PZnO and GZnO

Table 2. Functional group of PZnO and GZnO

S. No	Sample Name	Wave Number(cm-1)						
		O-H Stretching Vibration (free)	O-H Stretching Vibration (bonded)	C-H Stretching Vibration	N=O Stretching Vibration	N-H Stretching Vibration	O-H Stretching Vibration (free)	O-H Stretching Vibration (bonded)
1.	GZnO	2320	3960.65	2809.44	1450.49	3446.81	2320	3960.65
2.	PZnO	2318.44	3861.49	2800.64	1450.47	3589.53	2318.44	3861.49

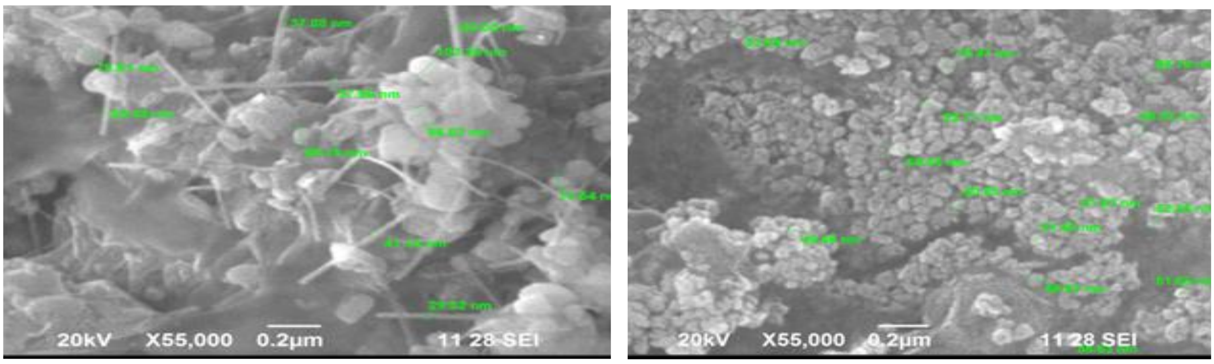

Fig. 4: SEM Performance of PZnO and GZnO

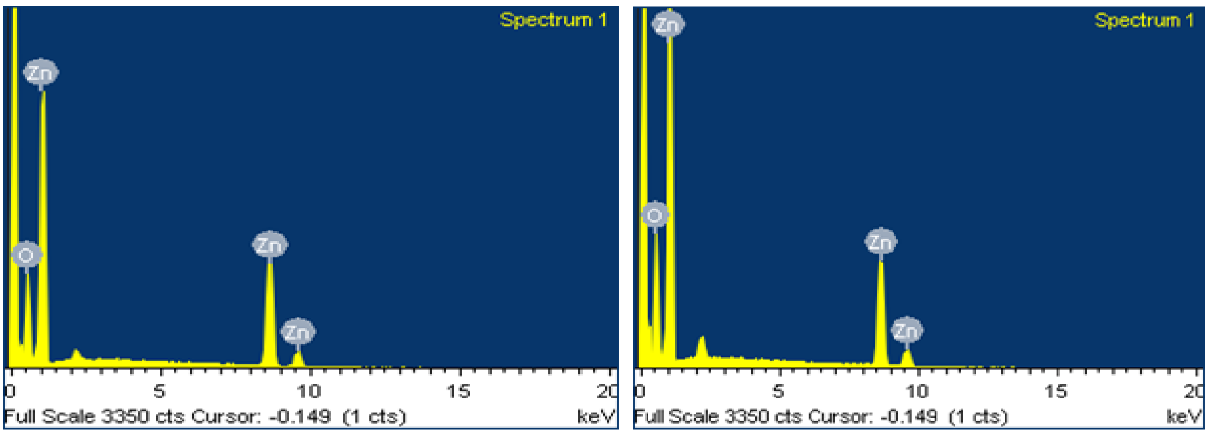

Fig. 5: EDAX Performance of PZnO (A) and GZnO (B)

The Energy Dispersive X-Ray Spectroscopy is used to investigate the elemental composition and chemical analysis of PZnO and GZnO. The analysis observed Zn (Zinc), O (Oxygen) for PZnO and GZnO. This represents the purity of the sample shown in (Figure 5). In, EDAX the presents of Zn and O reveals the capping agent in the sample.

4. CONCLUSION

Zinc oxide nanoparticles were synthesized by chemical and green synthesis method. XRD analysis predicts the crystalline size, lattice parameter and unit cell volume of the sample. The morphological structure was revealed by SEM. EDAX analysis the elemental composition of the sample. Thus the synthesized samples can be applied in the field of medicine for cancer treatment and as a water purifier in Environmental science.

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